

weighed and mixed in a V-shaped mixer to give a mixture having the above composition. The resulting herb mixture was processed into powder in a mill (power mill P-7 model, product of SYOWA chemical machine manufactory corporation) and then shaped into tablets using a tablet machine (product of HATA manufactory corporation), thereby preparing an ATP synthesis activator.

#### Test Example A

In this test example, the ATP synthesis activator prepared as stated above was used to perform an activation test for ATP synthesis in a human body. The test was performed on five men of ages 48 to 62 (average age: 55).

In this test, each of the above five men was orally administered with the ATP synthesis activator, twice a day, at a daily dose of 10 g for 3 consecutive days, along with 100 cc mineral water.

ATP levels were determined before and 3 days after starting the administration of the ATP synthesis activator by using an ATP detector HACCP-LIGHT38 (Microtec Co., Ltd.). Prior to ATP detection, the men brushed their teeth and then washed their mouths out three times. Subsequently, they further washed their mouths out with a commercially available mineral water that had been found to contain no ATP. About 20 ml of mineral water from each man was transferred into a clean cup for use as a sample. An aliquot (100  $\mu$ l) of each sample was taken into a detection tube and mixed with two drops of a luminescent reagent specifically prepared for the detector to determine ATP level of each sample using the detector.

Fig. 1 shows a comparison of ATP levels between before and 3 days after the administration of the ATP synthesis activator. Although some individual differences were found in ATP levels, as shown in Fig. 1, the average ATP level was 11615 RLU before administration, while the average ATP level was 16319 RLU after administration,

thereby indicating a significant difference before and after administration. This result indicates that the administration of the ATP synthesis activator results in an improved ATP synthesis activity in a human body.

#### Test Example B

In this test example, the ATP synthesis activator prepared as stated above was evaluated for its oxidation-reduction property. First, a rusted 10-yen coin made of copper and a solution containing 10 tablets of the ATP synthesis activator (400 mg per tablet) in 100 cc tap water (Minato-ku, Tokyo, Japan) were prepared. Next, the 10-yen coin was contacted with the above solution on one surface of the coin and then allowed to stand for 20 hours. After 20 hours, the surface of the coin contacted with the solution was observed, thereby indicating that the copper coin recovered its original luster after contacting with the ATP synthesis activator for 20 hours, whereas the coin was fully rusted at the time of starting the test.

In addition, tap water, prior to preparation of the solution, was measured for its oxidation-reduction potential, which was then used as a correction potential. The solution was measured for its pH and oxidation-reduction potential before and 20 hours after contacting with the coin. In both cases, the oxidation-reduction potential was measured using an ORP meter (commercially available from Toa Electronics Ltd. under the trade name of RM-12P) and the pH was measured using a pH meter (commercially available from HANNA under the trade name of PICCOLO HI 1280). The results are shown in Table 1.

**Table 1**

|                | Water temp. | Correction potential | Meter potential | Oxidation-reduction potential | pH  |
|----------------|-------------|----------------------|-----------------|-------------------------------|-----|
| Before         | 23°C        | +208 mV              | +268 mV         | +476 mV                       | 7.1 |
| 20 hours after | 23°C        | +208 mV              | -526 mV         | -318 mV                       | 7.4 |

Table 1 shows that the oxidation-reduction potential changes from +476 mV (at the time of starting the test) to -318 mV (after 20 hours), while the pH changes from 7.1 to 7.4. This suggests that the use of the above solution permits rust removal even at a neutral pH and that when dissolved in water, the ATP synthesis activator provides electrons in the aqueous solution over the course of time, thereby resulting in the reduction of rust formed on metal.

### Test Example C

The ATP synthesis activator was examined for its antitumor effect in a mouse model with Colon 26 carcinoma.

An 8-week-old BALB/C male mouse (microbiological grade: SPF) was purchased from CLEA Japan, Inc. for use in the preparation of a mouse model with Colon 26 carcinoma. This mouse was implanted with mouse colon carcinoma cells (Colon 26) by subcutaneous administration in the abdomen to prepare a mouse model with Colon 26 carcinoma. The mouse colon carcinoma cell line, Colon 26, is derived from the carcinoma cell line that has been established by repeating the intrarectal administration of N-methyl-N-nitroso-uretan to a BALB/C mouse.

On day 21 after the subcutaneous implantation, a tumor block was removed from the mouse with Colon 26 carcinoma and separated into individual cells, which were then suspended in sterilized physiological saline. The resulting suspension was subcutaneously administered to each of six 8-week-old BALB/C male mice in the abdomen at a tumor cell density of  $1 \times 10^5$  cells/100  $\mu$ l per mouse. Each mouse was bred under the following conditions:

|                                  |  |
|----------------------------------|--|
| Preset temperature and humidity: | 24 $\pm$ 1°C and 66 $\pm$ 6%                                       |
| Air conditioning system:         | 70% return-air system  |
| Lighting time:                   | 12-hour cycle under automatic control<br>(from 8:00 am to 8:00 pm) |

|                       |   |
|-----------------------|---|
| Breeding system:      | plastic cage                              |
| Feed:                 | sterilized CA-1 pellet (CLEA Japan, Inc.) |
| Water to be supplied: | distilled water                           |

In this test, the six mice thus prepared were divided into two groups of 3 mice, i.e., a control group and a test group. Each mouse in the test group was orally administered through a gastric tube with the ATP synthesis activator dissolved in distilled water at a dose of 200 mg/kg for 14 consecutive days. Each mouse in the control group was orally administered with distilled water. The administration of the ATP synthesis activator started the next day after preparing the mice with Colon 26 carcinoma. In both groups, each mouse implanted with tumor cells showed no visual or palpable change at the implanted site until 4 days after implantation. From 6 days after implantation, however, the mouse tended to show skin swelling in the abdomen associated with tumor cell growth and further showed emaciation and piloerection on day 14. In particular, a mouse with a large tumor volume showed decreased motility and sometimes crouched down on day 14.

After breeding for 15 days, each mouse was sacrificed by cervical dislocation and then assayed for its tumor volume and IL-12 and TNF- $\alpha$  levels. A vernier caliper (Plate Reader M-2300, NUNK) was used to determine a tumor volume according to the following equation: tumor volume ( $\text{mm}^3$ ) = (tumor length)  $\times$  (tumor width)<sup>2</sup>  $\times$  0.4. IL-12 and TNF- $\alpha$  levels were assayed as follows. The spleen was removed from each mouse and suspended in RPMI-1640 medium supplemented with 10% FC8. ConA was added to the resulting spleen cell suspension ( $2 \times 10^8$  cells/ml) at a concentration of 5  $\mu\text{g/ml}$ , followed by culturing at 37°C in a 5% CO<sub>2</sub> incubator (YAMATO) for 24 hours. The culture supernatant was then assayed for its IL-12 and TNF- $\alpha$  levels using a Cytoscreen kit (BIOSOURCE). These experiments were performed under air conditioning (70% return-air system) at a temperature of  $26 \pm 1^\circ\text{C}$  and at a humidity of  $66 \pm 6\%$ .

Fig 2 shows a comparison of tumor volumes between the ATP synthesis activator-administered group (test group) and the control group. Fig. 3 shows a comparison of IL-12 and TNF- $\alpha$  levels between the ATP synthesis activator-administered group (test group) and the control group.

Although some individual differences were found in tumor volumes, as shown in Fig. 2, the control group had an average tumor volume of 626.38 mm<sup>3</sup>, while the test group had an average tumor volume of 330.02 mm<sup>3</sup>, thereby indicating a significant difference between these two groups. This result indicates that the administration of the ATP synthesis activator provides an inhibitory effect on tumor volume increase.

As shown in Fig. 3, there was no significant difference in IL-12 (apoptosis inducer) levels between the test group and the control group. In contrast, a significant difference was found in TNF- $\alpha$  (tumor necrosis factor) levels between the test group (37.46 pg/ml) and the control group (20.45 pg/ml). These results suggest that the administration of the ATP synthesis activator provides a stimulating effect on TNF- $\alpha$  production in the mouse with Colon 26 carcinoma, thereby inhibiting tumor growth.

As stated above in detail, the ATP synthesis activator of the present invention can promote ATP synthesis in the body because it comprises, as an active ingredient, a mixture of a plurality of herbs having an ion-exchange capacity. Accordingly, the ATP synthesis activator can be used to ameliorate a symptom caused by lack of ATP.